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阿拉伯芥轉錄因子B-box domain protein 14參與光以及茉莉酸訊息傳遞路徑中的功能性研究

Functional studies of transcription factor B-box Domain Protein 14 involved in light and jasmonate signaling pathways in *Arabidopsis*

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中文摘要

光和植物激素茉莉酸（jasmonates）協同調控植物的生長和發育，涉及許多在這些途徑之中進行相互作用的基因。其中 FIN219（FAR-RED INSENSITIVE 219）在這兩條途徑中已有廣泛研究。通過對比在遠紅光（FR）下和有或無甲基化茉莉酸（MeJA）處理下的糖皮質激素誘導的 PGRFIN219（glucocorticoid-inducible FIN219），幼苗與 Col-0 的微陣列數據分析顯示，PGRFIN219 中的 BBX14 基因表現低於 Col-0，這暗示 FIN219 與 BBX14（B-BOX protein 14）之間可能存在潛在關係。然而 BBX14 在光和 JA 信號途徑中的作用尚未被了解，而且 HY5（ELONGATED HYPOCOTYL 5）和 COP1（CONSTITUTIVE PHOTOMORPHOGENIC 1） 也在光信號途徑中扮演關鍵角色。定量即時聚合酶鏈鎖反應分析（qPCR）顯示，FIN219 和 HY5 都影響 BBX14 的表現，同樣的 BBX14 也影響 FIN219 和 HY5 的表現。進一步的雙分子螢光互補（BiFC）分析顯示，BBX14 與 FIN219 及 HY5 之間存在相互作用，但未觀察到 BBX14 與 COP1 之間的相互作用。我們發現 MeJA 誘導的 BBX14 在黑暗條件下調控下胚軸長度；另外，BBX14 的過表現會在 FR 光下抑制 HY5 的功能。COP1 也位於 BBX14 的下游來調控下胚軸延長。總體而言，這些結果表明 FIN219、HY5 和 BBX14 可能是反饋迴路的一部分，並參與 JA 信號途徑中的其他機制，我們的研究發現顯示 BBX14 可能涉及光和 JA 信號途徑，以調控幼苗下胚軸的延長。

關鍵字：BBX14、FIN219/JAR1、光型態發生、茉莉酸、遠紅光

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Abstract

Light and plant hormone jasmonates (JA) coordinately regulate plant growth and development, involving numerous genes that participate in the crosstalk between these pathways. For example, FIN219 (FAR-RED INSENSITIVE 219) has been extensively studied in both pathways. Analysis of microarray data by comparing *PGRFIN219* (an inducible *FIN219* overexpression line) seedlings with Col-0 under far-red (FR) light with or without methyl JA (MeJA) treatment revealed lower expression of the *BBX14* (B-BOX protein 14) gene in *PGRFIN219* versus Col-0, suggesting a potential relationship between FIN219 and BBX14. However, the role of BBX14 in light and JA signaling pathways remains unexplored. Additionally, HY5 (ELONGATED HYPOCOTYL 5) and COP1 (CONSTITUTIVE PHOTOMORPHOGENIC 1) play crucial roles in light signaling pathway. Quantitative real-time PCR analysis shows that both FIN219 and HY5 affect *BBX14* expression. Similarly, BBX14 also affects the expression of *FIN219* and *HY5*. Further BiFC assays demonstrated the interaction between BBX14 and FIN219, as well as HY5, but no interaction between BBX14 and COP1 was observed. Furthermore, we found that BBX14 induction by MeJA regulates hypocotyl length under the dark conditions. Our results indicate that *BBX14* overexpression results in a suppression of HY5 function under FR light. COP1 is also in the downstream of BBX14 in regulating hypocotyl elongation. The feedback regulation

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between FIN219 and BBX14 influences the sensitivity of BBX14 response to MeJA in Arabidopsis. Overall, these results suggest that FIN219, HY5, and BBX14 may be part of a feedback loop and involved in other mechanisms in the JA signaling pathway. Our findings indicate that BBX14 is likely involved in both light and JA signaling pathways.

KEYWORDS: BBX14; FIN219/JAR1; Photomorphogenesis; Jasmonate; Far-red light

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Introduction

Light and plant hormone jasmonates regulate plant growth and development

Plants sense changes in their environments, such as light exposure, which triggers photomorphogenesis. They also detect abiotic and biotic stress, leading to the production of the plant hormone jasmonates (JA). JA include jasmonic acid and its derivatives, including methyl jasmonate (MeJA) and the JA-isoleucine (JA-Ile). These compounds help plants adapt and respond to various environmental cues by regulating gene expression and controlling various aspects of plant growth and development (Ruan et al., 2019). Light plays a crucial role in regulating many stages of plant growth, including seed germination, photomorphogenesis, shade avoidance, photosynthesis, circadian rhythms, and flowering duration (Paik and Huq, 2019). When seedlings are exposed to light, they undergo extensive morphological changes, characterized by the expansion of cotyledons and the shortening of hypocotyls. Conversely, under dark conditions, cotyledons close, and hypocotyls elongate. During seed germination, light affects whether seedlings exhibit a photomorphogenic or skotomorphogenic phenotype.

The regulatory network of the JA signaling pathway

In contrast, JA is also involved in plant growth and development, such as regulating inhibition of seed germination, delay of flowering, inhibition of the root growth and

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hypocotyl elongation (Ghorbel et al., 2021; Susila et al., 2023). The COI1-JAZ signaling module controls hypocotyl elongation in the JA pathway. Previous studies showed that COI1 suppressed hypocotyl elongation, whereas JAZ proteins, such as JAZ4, increase hypocotyl elongation (Chen et al., 2013; Oblessuc et al., 2020). When plants perceive biotic or abiotic stresses, they produce jasmonic acid (JA). The biosynthesis of JA is initiated, leading to the activation of the FAR-RED INSENSITIVE 219 (FIN219/JAR1) enzyme, which is responsible for converting JA into JA-Ile. FIN219/JAR1 then interacts with the JA receptor protein CORONATINE INSENSITIVE 1 (COI1), facilitating the ubiquitination and subsequent degradation of JAZ proteins by the 26S proteasome (Ruan et al., 2019). JAZ proteins are negative regulators of JA-responsive genes. Their degradation results in the release and activation of downstream genes. MYC2, which can interact with most JAZ proteins, plays a key role in activating JA-mediated responses. MYC proteins are activators of many JA-mediated responses and are targets of JAZ repressors, ultimately leading to the activation of the JA signaling pathway (Antico et al., 2012). When JAZ proteins degrade, they liberate MYC2 and ethylene response factors (ERFs)/octadecanoid-responsive Arabidopsis 59 (ORA59). These factors then initiate the expression of JA-mediated defense genes, such as PDF1.2 and VSP1 (Cui et al., 2021). This mechanism helps plants face various biotic and abiotic stresses in their natural environment.

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Role of FIN219 and COP1 in light signaling pathways and photomorphogenic development

In addition to the involvement of COI1-JAZ in plant hypocotyl development, FIN219 also plays a role in the light signaling pathway. FIN219 acts as a suppressor of CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), a crucial regulator of seedling skotomorphogenic development. Under far-red (FR) light conditions, phytochrome A (phyA) is converted to its active Pfr form and, with the addition of MeJA, undergoes phosphorylation. This activated phyA antagonizes FIN219. Subsequently, FIN219 enhances the association of COP1 in the cytoplasm, likely facilitating an interaction between photoactive phyA and related photomorphogenic factors. This interaction initiates the expression of downstream genes related to the photomorphogenic development (Jiang et al., 2023). Additionally, the presence of MeJA affects COP1 as well; under dark conditions, MeJA causes COP1 to accumulate in the cytoplasm due to the interaction between FIN219 and COP1, which suppresses COP1's function in the nucleus (Wang et al., 2011).

Regulatory networks involving COP1 and BBX factors in photomorphogenesis

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In the dark, COP1 enters the nucleus and targets positive regulators, such as ELONGATED HYPOCOTYL 5 (HY5), HY5 HOMOLOGUE (HYH), and LONG AFTER FAR-RED LIGHT 1 (HFR1) for the ubiquitination and subsequent degradation of these proteins by the 26S proteasome, thereby preventing photomorphogenic development of the seedlings (Osterlund et al., 2000). Several B-box (BBX) transcription factors are also affected by COP1. COP1 suppresses the function of BBX4, BBX20, BBX21, and BBX22. However, BBX21 and BBX22 directly interact with HY5 and enhance its activity, thereby promoting photomorphogenesis in seedlings (Gangappa and Botto, 2014).

Structural and functional diversity of B-box (BBX) proteins in Arabidopsis

The B-box (BBX) proteins are a class of zinc-finger transcription factors that contain a B-box domain with one or two B-box motifs, and sometimes also feature a CCT (CONSTANS, CO-like, and TOC1) domain. BBX proteins are grouped into five structural groups depending on the presence of at least one B-box domain with or without a CCT domain. The B-box domain is often associated with proteins that contain RING and coiled-coil domains. The CCT domain was identified in CONSTANS (CO), CO-LIKE, and TIMING OF CAB1 (TOC1) proteins in *Arabidopsis thaliana*, all of which act as critical regulators of flowering (Gangappa and Botto, 2014; Talar and

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Kiełbowicz-Matuk, 2021). CO was the first B-box protein identified in *Arabidopsis*, which contains two B-box domains and a CCT domain, is a central regulator of flowering time. CO promotes flowering in response to long days by activating the expression of *FLOWERING LOCUS T* (*FT*).

Cross-talks between light and JA signaling pathways are mediated by BBX, MYC2, and HY5

Previous studies demonstrated the involvement of a great number of B-box (BBX) transcription factors in both light and hormone signaling pathways. For example, in sweet potato, BBX protein IbBBX24 modulates the transcription of IbJAZ10 and IbMYC2, inhibiting IbJAZ10 to release IbMYC2 and initiates JA signaling (Zhang et al., 2020). Additionally, BBX20, BBX21, and BBX22 promote the expression of *HY5*, a bZIP transcription factor, thereby inhibiting hypocotyl growth. Conversely, BBX24, BBX25, and BBX29 inhibit HY5 activity, thus regulating seedling photomorphogenesis (Gangappa and Botto, 2014). HY5 and MYC2 play pivotal roles in regulating hypocotyl growth, demonstrating an antagonistic relationship in this process. The JA signaling pathway intersects with the light signaling pathway through shared downstream regulatory proteins such as MYC2 and HY5 (Prasad et al., 2012).

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Structural and functional profiling of BBX14 in *Arabidopsis*

B-BOX protein 14 (BBX14) belongs to the B-box transcription factor family, which comprises 32 members in *Arabidopsis* and belongs to group III. *Arabidops*is has five groups, all of which have at least one conserved B-box domain. BBX14 shares structural similarities with BBX15, BBX16, and BBX17, including the presence of two B-box domains and a CCT domain, suggesting potential functional similarities among these proteins. Several studies have highlighted the regulatory roles of BBX14 and BBX15 in the GLK1 signaling pathway, specifically in the processes, such as chlorophyll biosynthesis. Furthermore, BBX14 is known to be involved in high light (HL)-induced expression in the JA signaling pathway (Tikkanen et al., 2014; Atanasov et al., 2024). BBX14, BBX15, and BBX16 interact with the CONSTANS (CO) protein in the nucleus, and their expression inhibits CO-mediated transcription of the *FT* (*Flowering Locus T*) gene, leading to a delayed flowering (Susila et al., 2023).

The unpublished microarray data suggests that FIN219 may regulate BBX14 functions in light and JA signaling pathways. Compared to other members in the group IV of the BBX family, less information is available regarding BBX14's functions in plant hormone and jasmonate signaling pathways.

Objective: investigating the role of BBX14 in integrating light and jasmonate

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signaling pathways

Overall, the connection among FIN219, COP1, and HY5 is largely clear. Each has its own function: FIN219 is an enzyme responsible for converting JA into JA-isoleucine, which acts as a suppressor of COP1, thereby preventing a skotomorphogenic development in seedlings. COP1 is an E3 ubiquitin ligase that degrades light-responsive transcription factors such as HY5. HY5 promotes positive photomorphogenesis in seedlings and is regulated by FIN219. However, the molecular mechanism underlying the integration of light and JA signaling pathways involving BBX14 is still unclear. Therefore, this study aims to investigate the role of BBX14 in the light and JA signaling pathways by exploring its relationship with FIN219, HY5, and COP1.

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Materials and Methods

Plant materials and growth conditions

The *Arabidopsis* materials used in this study are wild-type Columbia (Col-0), the mutants *bbx14, hy5, cop1-6* and *fin219-2,* and overexpression lines *PGRFIN219* and *PGRHY5*. The *bbx14* mutant (SAIL_1221_D02), with a T-DNA insertion at the 3' UTR, was obtained from ABRC. The *hy5* mutant (*hy5ks50*) has a T-DNA concatemer insertion, accompanied by a deletion between positions t-147 and t-935 (Oyama et al., 1997). McNellis et al. (1994) reported that the *cop1-6* allele was a weak mutant of COP1. The *fin219-2* (SALK_059774) has a T-DNA inserted in the second exon. The *PGRFIN219* and *PGRHY5* overexpression lines are glucocorticoid/DEX-inducible lines in *fin219-2* and *hy5* mutant backgrounds, respectively (Wang et al., 2011).

Seeds were surface sterilized with 30% (v/v) bleach and 0.02% (v/v) Triton X-100 for 5 min, washed three times with sterile water, and sown on Murashige and Skoog (MS) medium supplemented with 0.9% (v/w) agar and 0.7% (w/v) sucrose for phenotypic examination. The seeds were stratified in darkness at 4°C for 3 days before transferred to a designated light source.

Construction of plasmids

The full-lengthes of *BBX14*, *BBX30*, or *BBX32* coding sequences were cloned into the

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pCR8 vector and subsequently introduced into the pEarley Gateway 201YN and pEarley Gateway 202YC vectors (Walter et al., 2004) driven by the 35S promoter using the Gateway LR Clonase enzyme mix. The full-length BBX14 open reading frame was also cloned into the SalI/PstI sites of the pCR8 vector and introduced into the pEarley Gateway 101 vector. The pEarley Gateway 101 constructs were transformed into *Agrobacterium tumefaciens* GV3101, and into Col-0, *hy5*, *PGRFIN219* (glucocorticoid-inducible FIN219), and *PGRHY5* (glucocorticoid-inducible HY5) plants with the floral-dip method (Clough and Bent, 1998) to generate different backgrounds of the *BBX14* overexpression line. Transgenic plants were selected on MS medium containing 10 mg/L phosphinothricin.

Measurement of hypocotyl length

To measure the hypocotyl length of *Arabidopsis* seedlings, seeds were sown on plates and stratified at 4°C in darkness. The seeds were then exposed to continuous white light for 12 hours for uniform germination. Subsequently, the seeds were transferred to a designated light source and incubated at 22°C for 3 days. Three-day-old seedlings from various light treatments were lain on plates and photographed. Hypocotyl lengths were measured using ImageJ software.

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RNA extraction and quantitative real-time PCR

Total RNA was extracted from frozen plant materials using a LabPrepTM RNA Kit according to the manufacturer's protocol. RNA was quantified using a NanoDrop (Thermo Scientific). A total of 1 μg of total RNA was used per sample for cDNA synthesis using random primers and reverse transcriptase according to the manufacturer's protocol. cDNAs were then diluted and used as templates for qPCR using SYBR Green Supermix reagent (Bio-Rad) in a Bio-Rad CFX96 real-time system. All PCRs were performed with preincubation for 3 minutes at 95 °C, followed by 40 cycles of denaturation at 95 °C for 10 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 30 seconds. All expression levels were quantified relative to the housekeeping gene *UBQ10*, and reactions were performed with triplicate for each cDNA sample. Designed primers and probes used are listed in Supplemental Tables 1.

Bimolecular fluorescent complimentary (BiFC) assay

For BiFC experiments, the coding sequences, without the termination codon, were cloned into the pEarleyGate-201 YN and pEarleyGate-202 YC. Then, *A. tumefaciens* strain GV3101 harboring the constructs was transiently co-expressed in all possible combinations of pEarleyGate-201 YN or pEarleyGate-202 YC fusion proteins in *Nicotiana benthamiana* leaves. The cultured cells were diluted to an optical density

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(OD) of 0.2, mixed, and then injected into the leaves. After 48 hours of recovery, the plants were exposed to light for 12 hours before confocal imaging. GFP and NLS-mCherry expression of the nuclear localized fluorescence was observed using a Nikon® H600L confocal microscope with a DS-Ri2 camera.

Generation of the double mutants

The *bbx14* mutant was used as the male parent and crossed with the *fin219-2*, *hy5*, and *cop1-6* mutants, which served as the female parents, to generate the double mutants. Additionally, the *BBX14* overexpression line was used as the male parent and crossed with the *fin219-2* mutant to produce the *BBX14* overexpression line in the *fin219-2* background.

To confirm these mutant materials, I extracted the genomic DNA from adult leaves with 50 μL of DNA extraction buffer and incubated for 5 minutes, followed by 500 μL of double-distilled water to stop the reaction. Then add 1 μL of the solution to the PCR reaction. Besides, the overexpression transgenic lines used in this study are all homozygous at the third generation.

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Results

BBX gene expression profiles in *fin219-2* mutants under far-red light and MeJA treatments

Previous studies showed that BBXs were involved in various plant hormone and light signaling pathways (Gangappa and Botto, 2014). Whether some BBXs participate in the jasmonate signaling pathways remains unclear. It was known that FIN219 affected many genes, including transcription factors and regulators involved in light and jasmonates (JA) signaling. Therefore, our first objective is to determine how FIN219 affects the *BBX* genes based on microarray data in response to far-red (FR) light and JA treatment.

In the microarray data, comparing the *PGRFIN219* with Col-0 under FR light, we observed that the expression levels of *BBX11, 14, 15, 16, 19, 20, 27, 28, 29,* and *31* genes were downregulated. In the *fin219-2* mutant compared to Col-0, the expression of *BBX16, 31,* and *32* were downregulated. Additionally, when MeJA was added to the *fin219-2* mutant compared to Col-0, *BBX31* and *32* were downregulated, whereas *BBX6*, *17*, and *29* were upregulated, respectively. Taken together, these data imply that these BBXs are likely regulated in response to FR light and MeJA by FIN219 (Table S1).

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Regulation of *BBX14*, *BBX31*, and *BBX32* genes expression by FIN219, HY5, and COP1 in response to light and MeJA treatments

Further investigation into the regulatory relationship between FIN219 and BBX proteins focuses on several specific BBX genes. Previous studies revealed that these genes might be involved in light and plant hormone signaling pathways. We have selected three candidate BBX genes, BBX14, BBX31, and BBX32. To clarity the roles of these candidate genes B*BX14, BBX31,* and *BBX32* in the interactions among FIN219, HY5, and COP1, we have decided to study the expression levels of these transcription factors in *fin219-2, hy5* and *cop1-6* three mutants and inducible overexpression lines *PGRFIN219* and *PGRHY5* under far-red light and dark conditions. Based on our microarray data, this experimental design will further validate and expand our findings.

BBX14 is bound by GLK1, enhancing high light tolerance and is involved in high light-induced expression in the JA signaling pathway (Tikkanen et al., 2014; Atanasov et al., 2024). The expression levels of *BBX14* in Col-0 were significantly induced by MeJA under dark conditions. In the *hy5* mutant, *BBX14* expression increased with MeJA treatment, whereas in the *PGRHY5* line, *BBX14* expression showed the opposite pattern under dark conditions (Figure 1A). Additionally, *BBX14* expression decreased in the *PGRFIN219* line compared to the wild type under dark conditions. Under FR light, *BBX14* expression levels decreased in both the *PGRFIN219* and *PGRHY5* lines

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compared to Col-0 (Figure 1B). Taken together, these results indicate that *BBX14* is regulated by FIN219 and HY5 under dark and far-red light conditions and participates in the JA signaling pathway in the dark.

Under high doses of UV-B radiation, BBX31 promotes the accumulation of UV-protective flavonoids and phenolic compounds. HY5 enhances BBX31 transcription under UV-B light by directly binding to its promoter. In visible light, HY5 binds to the G-box cis-element in the promoters of BBX31, negatively controlling its transcription levels. Transgenic seedlings overexpressing BBX31 exhibit elongated hypocotyls (Heng et al., 2019; Yadav et al., 2019). *BBX31* gene expression levels were dramatically increased in *cop1-6* mutant under dark conditions. However, its expression levels also increased in the *hy5* and *cop1-6* mutants compared to the Col-0 under far-red light. Notably, there was no significant difference in *BBX31* expression with or without MeJA treatment under FR light conditions (Figures 1A and B). These results indicate that COP1 likely regulates *BBX31* expression in response to MeJA under dark conditions. *BBX31* is regulated by HY5 and COP1, but not induced by MeJA under FR light.

BBX32 plays a role in light signaling, as seedlings overexpressing BBX32 display elongated hypocotyls along with reduced cotyledon expansion, due to BBX32 negatively regulating light signaling and promoting BR signaling to inhibit cotyledon opening (Holtan et al., 2011; Ravindran et al., 2021). In addition, *BBX32* gene

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expression levels were dramatically increased in the *cop1-6* mutant and *PGRFIN219* line under dark conditions. Additionally, *BBX32* expression levels were elevated in the *cop1-6* mutant under FR light. Interestingly, *BBX32* expression in the *cop1-6* mutant with or without MeJA treatment showed opposite patterns under darkness and FR light conditions (Figures 1A and B). These results indicate that COP1 likely regulates *BBX32* expression in response to MeJA under dark and far-red light conditions. *BBX32* is regulated by FIN219 as well under dark conditions.

Overall, *BBX14* is regulated by FIN219 and HY5 under darkness and FR light conditions. *BBX31* and *BBX32* are regulated by COP1 under darkness and FR light conditions and *BBX31* by HY5 under FR light conditions; and *BBX32* also by FIN219 in the dark. These findings suggest that *BBX14, BBX31*, and *BBX32* play vital roles in integrating light and JA signaling to regulate the seedling growth.

The candidate genes *BBX14*, *BBX31*, and *BBX32* were tested for interactions with FIN219 under white light conditions

In addition to validating the relative gene expression levels of *BBX14, BBX31*, and *BBX32* in different mutants and inducible lines, we aim to examine the interaction of these candidate genes with FIN219 at the protein level. This is important because many BBX proteins interact with HY5 and COP1, influencing the regulation of the seedling

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photomorphogenesis (Gangappa and Botto, 2014). Notably, FIN219 plays a key role in integrating light and JA signaling pathways. At the protein level, COP1 degrades HY5 in the dark, while FIN219 suppresses COP1. FIN219 negatively regulates COP1 levels through physical interaction, thereby stabilizing the HY5 protein (Wang et al., 2011).

To further investigate possible physical interaction between FIN219 and BBX14, BBX31, and BBX32 under white light conditions, we utilized constructs of the full-lengths of FIN219, BBX14, BBX31, and BBX32. The sequences encoding the N-terminal or C-terminal fragments of yellow fluorescence protein (YFP) were driven by the 35S promoter in our experiments. Our observations showed that YFP fluorescence was observed for BBX14 interacting with FIN219 in the nuclear compartment of transformed cells, but not for BBX31 or BBX32 (Figure 2A). These results demonstrate that BBX14, but not BBX31 or BBX32, can interact with FIN219 under white light conditions.

BBX14 interaction with FIN219, HY5, and COP1 by BiFC analysis under different light conditions and MeJA treatments

Based on the RT-qPCR analysis and BiFC data, we discovered that the expression levels of *BBX14* were induced by MeJA under dark conditions, and *BBX14* may be regulated by HY5 and FIN219. The BiFC was further used to examine the interaction between FIN219 and BBX14 under specific light conditions. These results indicated that BBX14

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participates in the FIN219-associated signaling pathways. Consequently, we prioritize BBX14 as a key transcription factor for further investigation on the roles of FIN219, HY5, and COP1. To confirm the interaction between BBX14 and FIN219 under different light conditions and in the presence of MeJA, we co-expressed BBX14YN and FIN219YC in the leaves of *N. benthamiana* under the blue light, FR light and dark condition (Figures 3B-D). Additionally, we treated the leaves with 50 μM MeJA to observe whether this exogenous application affects their interaction and localization, either in the nucleus or the cytoplasm, or alters their ability to interact.

Previous study showed that under FR light and MeJA treatment conditions, PHYA and FIN219 interacted in the cytoplasm before translocating into the nucleus (Jiang et al., 2023). The results showed that BBX14 interacted with FIN219 in the nucleus under FR light, blue light, and dark conditions, irrespective of the presence of MeJA (Figures 3B-D). We next co-expressed BBX14-YN and HY5-YC (Figures 4A-C), and found that BBX14 interacted with HY5 in the nucleus under FR light and dark conditions, regardless of MeJA presence (Figures 4B and C). However, when we co-expressed COP1-YN and BBX14-YC (Figures 5A-C), BBX14 did not interact with COP1 in either the nucleus or the cytoplasm under FR light and dark conditions, irrespective of MeJA (Figures 5B and C). These findings demonstrate that BBX14 interacts with FIN219 and HY5, but not with COP1.

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Functional roles of BBX14 in modulating hypocotyl length under various light and JA treatments

In the previous results, we investigated the molecular interactions of BBX14 and FIN219, HY5, as well as COP1. Next, we aim to explore how these molecular interactions affect the physiological responses and phenotypic changes.

To examine the functions of BBX14 in light and JA signaling, we used *bbx14* mutant and *BBX14* overexpression lines in Col-0 background under the different light conditions. We did not observe significant differences in the hypocotyl lengths between wild type and *bbx14* mutant under far-red light, blue light, and darkness (Figures 6A-C). The 35S: *BBX14* seedlings did not show any difference in the hypocotyl lengths compared to Col-0 under blue light, with or without 50 μM MeJA treatment. However, 35S: *BBX14* seedlings displayed shorter hypocotyl lengths than Col-0 under FR light without MeJA. Additionally, although there was no difference in hypocotyl length between Col-0 and 35S: *BBX14* seedlings without MeJA treatment in darkness, the hypocotyl length was shorter in the 35S: *BBX14* seedlings #3 and #24 lines than Col-0 with MeJA treatment. All these data together indicate that the *BBX14* overexpression line is more sensitive under FR light without JA and more sensitive to JA-mediated inhibition of hypocotyl elongation in darkness.

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Regulation of hypocotyl length by BBX14 and FIN219 under various light conditions

Next, we further explored how BBX14 and FIN219 control hypocotyl lengths under different light conditions. We generated a 35S:*BBX14* overexpression line in *fin219-2* mutant background by crossing a 35S:*BBX14* transgenic line in the Col-0 background with *fin219-2* mutant. Additionally, we generated a *BBX14* overexpression line in the *PGRFIN219* background by using the floral dip method. We also generated a double mutant by crossing *fin219-2* mutant with *bbx14* mutant.

The results showed that under FR light conditions, the *fin219-2* mutant exhibited the greatest elongation of hypocotyl length (Figure 7A). The *fin219-2* and *bbx14* double mutant displays a phenotype similar to the *fin219-2* single mutant, suggesting that FIN219 might act downstream of BBX14 to regulate its function. Interestingly, the overexpression of *BBX14* in the *fin219-2* mutant (*BBX14*/*fin219-2)* and the *PGRFIN219* backgrounds (*BBX14*/*PGRFIN219)* resulted in a hypocotyl length similar to that of Col-0. The *BBX14*/*fin219* and *BBX14*/*PGRFIN219* are also similar to the *fin219-2* mutant in the presence of MeJA under FR light, suggesting that BBX14 may suppress FIN219 functions in response to MeJA. It is possible that their interaction in regulating

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hypocotyl length involves another mechanism, such as a negative feedback mechanism in response to MeJA.

BBX14 influences the response to MeJA treatment under both dark and blue light conditions (Figures 7B and C). Specifically, under blue light, no significant differences were observed between the wild type, *bbx14* mutant, *fin219-2* single mutant, and the double mutant. Additionally, BBX14 overexpression in *fin219-2* and *PGRFIN219* backgrounds did not significantly differ from Col-0. Similarly, the double mutant and *BBX14*/*PGRFIN219* exhibited a phenotype similar to the *fin219-2* mutant in the presence of MeJA, suggesting that BBX14 suppresses *PGRFIN219* phenotype under blue light. Under dark conditions, the double mutant *fin219-2bbx14* resembled the *fin219-2* mutant, while *BBX14*/*fin219* mirrored both the *fin219-2* mutant and wild type. Moreover, in the *PGRFIN219* background under dark conditions, *BBX14*/*PGRFIN219* showed a phenotype similar to Col-0 and *PGRFIN219* without MeJA treatment. The presence of BBX14 affected the *PGRFIN219* phenotype, leading to longer hypocotyl length. These findings highlight that BBX14 may play a key role in regulating FIN219 functions, particularly in regulating JA homeostasis under light conditions such as FR and blue light, in response to MeJA. This involvement is crucial for controlling the JA biosynthesis and signaling pathway.

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Regulation of hypocotyl length by BBX14 and HY5 under different light conditions

Furthermore, we investigated how BBX14 and HY5 interaction regulates hypocotyl lengths under different light conditions. We generated a 35S:*BBX14* overexpression line in both the *hy5* mutant background and the *PGRHY5* background by using the floral dip method, respectively. We also generated the double mutant *hy5bbx14* by crossing the *hy5* mutant with the *bbx14* mutant.

Under FR light, the double mutant *hy5bbx14* and the *BBX14/hy5* line exhibited an intermediate phenotype between the *hy5* single mutant and Col-0. In contrast, the *BBX14/PGRHY5* overexpression line is similar to Col-0 (Figure 8A), suggesting that *BBX14* overexpression results in a suppression of HY5 function under FR light. The molecular mechanisms underlying this suppression remain elusive.

Under blue light with MeJA treatment, the *BBX14/hy5* line, and the *BBX14/PGRHY5* line is also similar to the *PGRHY5* line (Figure 8B). This reveals that *BBX14* overexpression can suppress the phenotype caused by the *hy5* mutation in the absence of MeJA.

Under dark conditions, the *BBX14/PGRHY5* line exhibits an intermediate phenotype between the *PGRHY5* line and Col-0, both with and without MeJA (Figure 8C). These intermediate phenotypes further support that BBX14 may regulate HY5 functions under darkness.

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Investigating the effects of BBX14 and COP1 integration on hypocotyl length

Furthermore, we investigated how BBX14 and COP1 regulate the hypocotyl length under different light conditions. We generated a 35S:*BBX14* overexpression line in the *cop1-6* mutant background (*BBX14/cop1-6)* by using the floral dip method. We also generated the double mutant *bbx14cop1-6* by crossing the *cop1-6* mutant with the *bbx14* mutant for further studies.

The double mutant *bbx14cop1-6* showed a hypocotyl phenotype similar to *cop1-6* under FR light, blue light and the dark conditions (Figures 9 A-C), suggesting that COP1 is in the downstream of BBX14 in the regulation of hypocotyl elongation. The *BBX14/cop1-6* did not show significant differences compared to the *cop1-6* mutant, with or without MeJA, under FR light, blue light, and dark conditions (Figures 9 A-C), which implies that COP1 is also in the downstream of BBX14 in regulating hypocotyl elongation.

BBX14 regulates *FIN219* and *HY5* transcription levels

Not only does HY5 control the downstream *BBX* genes, but BBXs also regulate *HY5* expression. Previous studies showed that BBX11 activates the transcription of *HY5* by directly binding to the HY5 promoter (Zhao et al., 2020). To further understand the

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involvement of BBX14 in JA and light signaling pathways, we will investigate the expression levels of FIN219 and HY5. We aim to clarify the role of BBX14 by determining whether BBX14 can regulate both *HY5* and *FIN219* expression.

The expression levels of *HY5* gene in the *bbx14* mutant increased under darkness (Figure 10 A) and FR light (Figure 10 B) conditions, suggesting that BBX14 negatively regulates *HY5* transcript levels under darkness and FR light condition, especially the darkness. However, *HY5* transcript level in the *bbx14* mutant did not show a significant difference compared to that in Col-0 with MeJA treatment in the dark (Figure 10A). In contrast, *HY5* transcript level was reduced in the *bbx14* mutant compared to Col-0 under FR light with MeJA treatment (Figure 10 B). Consistently, *BBX14* overexpression increased *HY5* transcript level in the presence of MeJA, and decreased *HY5* transcript levels in the absence of MeJA (Figure 10 B). Under dark conditions, the expression of the *FIN219* gene is not significantly different in both the *bbx14* mutant and the *BBX14* overexpression line, regardless of MeJA treatment (Figure 10 A and B). However, *FIN219* gene expression decreased in both the *bbx14* mutant and the *BBX14* overexpression line compared to Col-0 under FR light conditions. These results demonstrate that BBX14 may modulate *HY5* and *FIN219* transcript levels, potentially through known BBX14 regulatory pathways or interactions with these genes.

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Discussion

Exploring the potential BBX14 binding sites on the HY5 promoter

Quantitative real-time PCR analysis showed an increased relative expression of *HY5* in the *bbx14* mutant, suggesting that BBX14 may directly bind to the *HY5* promoter (Figure 10). Previous study indicated that several BBX proteins, such as BBX11, BBX21, and BBX23, directly bind to the G-box cis-element in the *HY5* promoter to activate HY5 expression in *Arabidopsis*, promoting photomorphogenesis (Zhang et al., 2017; Zhao et al., 2020). In other species like tomato and rice, similar interactions have been observed. In rice, OsBBX14 binds to the T/G-box of the promoter, acting as a positive regulator in the light signaling pathway. In tomato, SlBBX20 and SlBBX21 interact with *Sl*HY5 and bind to the G-box promoter to activate its transcription, forming a feedback loop that inhibits its own gene transcription under UV-B (Bai et al., 2019; Yang et al., 2022).

Although previous studies showed that BBXs bind to the G-box, it is important to note that the *HY5* promoter also contains ACEs and CCAAT box cis-elements (Lescot et al., 2002). BBX20, BBX21, and BBX22 have been shown to bind to ACEs in the *MYB12* and *F3H* promoter regions, enhancing gene expression involved in photomorphogenesis and secondary metabolism (Bursch et al., 2020). Additionally, BBX1 (CO) binds to the CCAAT box on the FLOWERING LOCUS T (FT) promoter

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(Tiwari et al., 2010).

Given these findings, BBX14 may bind to multiple sites, including the G-box, ACEs, and CCAAT box, of the *HY5* promoter. To confirm whether BBX14 directly binds to the *HY5* promoter, further experiments such as Electrophoretic Mobility Shift Assay (EMSA) or Chromatin Immunoprecipitation (ChIP) are needed.

Investigating the effects of MeJA on BBX14, FIN219, and HY5 interactions

In the BiFC data, we discovered that FIN219 and HY5 interact with BBX14 regardless of the presence of MeJA (Figures 3 and 4). As mentioned in the results, MeJA may facilitate translocation. Previous studies indicated that FIN219 interacted with COP1, and MeJA treatment enhances their interaction under dark and FR light conditions. MeJA also enhances phyA and FIN219 interaction under FR light and dark conditions. Moreover, MeJA treatment enhances the interaction between FIN219 and the C terminus of CRY1 (GUS-CCT1) under blue light (Chen et al., 2018; Jiang et al., 2023).

In the BiFC assay, detecting changes in fluorescence intensity with MeJA treatment is difficult. Therefore, our next step involves performing Co-Immunoprecipitation (Co-IP) experiments to investigate how the interaction between FIN219 or HY5 and BBX14 is influenced by the presence or absence of MeJA, including potential changes in the

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interaction intensity. Furthermore, we will examine the interaction involving BBX14 and conduct pull-down assays to validate these interactions.

BBX31 and BBX32 have potential functions in the light and jasmonic acid pathways

Previous studies showed that HY5 protein binds to the promoter region of *BBX31*, repressing its transcription. BBX31 is a direct and negative target of *HY5* (Heng et al., 2019). Our quantitative real-time PCR analysis shows that *BBX31* expression levels are increased in the *hy5* and *cop1-6* mutants compared to the wild type under far-red light (Figure 1B). Although *BBX31* gene expression in the *hy5* mutant is not affected by MeJA, the *cop1-6* mutant shows different expression levels with or without MeJA compared to Col-0 under both light conditions (Figure 1B). These results suggest that BBX31 may be involved in jasmonic acid pathway in a COP1-dependent manner.

Previous studies primarily focused on the role of BBX32 in flowering time regulation (Tripathi et al., 2017). While it has been reported that BBX32 is involved in the BBX21 and HY5 dependent pathways, there was no direct interaction detected between BBX32 and HY5. This suggests that BBX32 likely acts as a part of a larger protein complex modulating the HY5 complex response to light signal transduction (Holtan et al., 2011).

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Our quantitative real-time PCR analysis did not show significant changes in *BBX32* expression in the *hy5* mutant or in *PGRHY5* compared to the Col-0 (Figure 1, A and B). However, *BBX32* expression was dramatically increased in the *cop1-6* mutant. These results indicate that COP1 likely plays a significant role in regulating *BBX32* expression to MeJA under dark and FR light conditions. This suggests that BBX32 might not directly interact with HY5, it could still be a part of a broader protein complex modulating the HY5 complex response to light and JA signal transduction.

Our quantitative real-time PCR analysis did not show significant changes in *BBX31* and *BBX32* expression in the *fin219-2* mutant (Figure 1, A and B). However, the microarray data showed obvious differences compared to the Col-0 (Table S1). This discrepancy is likely because the microarray data only compared the *fin219-2* mutant with the Col-0, whereas my quantitative real-time PCR analysis also included comparisons with other mutants, making the differences between the Col-0 and the *fin219-2* mutant less apparent.

Feedback regulation between FIN219 and BBX14 influences MeJA sensitivity in *Arabidopsis*

Our data from quantitative real-time PCR analysis shows that the abundance of one gene influences the expression of the other. When FIN219 levels are high, *BBX14*

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expression decreases, regardless of MeJA presence (Figure 1, A and B). Similarly, when BBX14 is absent or overexpressed, *FIN219* expression decreases with MeJA treatment under FR light condition (Figure 10B). These results suggest that the regulation between FIN219 and BBX14 may involve a feedback loop or other unknown mechanisms in the JA signaling pathway. Key components, such as LOX, AOC, and OPR3, which function upstream of FIN219 in JA biosynthetic pathway, may also be involved. Next, we will examine the expression of these genes to determine if BBX14 affects other JA biosynthetic genes, consequently influencing *FIN219* expression.

Next, we discuss the interaction between FIN219 and BBX14 in relation to hypocotyl length. Our data show that the *BBX14/fin219* and *BBX14/PGRFIN219* exhibit similar phenotypes to the *fin219-2* mutant in the presence of MeJA under FR light (Figure 7A). Since *PGRFIN219* shows a dwarf phenotype, *BBX14/PGRFIN219* exhibits a phenotype similar to Col-0, suggesting that *BBX14* overexpression suppresses *PGRFIN219* phenotype. Thus, it is critical to examine the regulatory relationship between their protein levels in response to FR light and JA.

Previous studies showed that FIN219 levels are regulated by a negative feedback mechanism in response to MeJA, which is involved in controlling JA biosynthesis and signaling pathways (Chen et al., 2015). This information suggests that BBX14 and FIN219 interaction may serve as a role in regulating JA homeostasis.

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Role of BBX14 induction by MeJA in hypocotyl length regulation under dark conditions

JA is also involved in suppressing hypocotyl elongation (Ghorbel et al., 2021; Susila et al., 2023). The COI1-JAZ signaling module controls the hypocotyl length in the JA pathway. Previous studies demonstrated that COI1 suppresses hypocotyl elongation, whereas JAZ proteins, such as JAZ4, increase hypocotyl elongation (Chen et al., 2013; Oblessuc et al., 2020).

Our quantitative real-time PCR analysis data show that the expression levels of *BBX14* were significantly induced by MeJA in the wild type under dark conditions (Figure 1A). Additionally, 35S: *BBX14* seedlings was shorter compared to the wild type with MeJA treatment under darkness. These data suggest that under dark conditions, BBX14 induction by MeJA increases seedling sensitivity to MeJA, resulting in a shorter hypocotyl.

HY5 and BBX14 interaction may regulate hypocotyl elongation

In the dark, HY5 suppresses *BBX14* expression regardless of MeJA compared to Col-0 (Figure 1A), suggesting that HY5 is a negative regulator of *BBX14* expression under dark conditions. However, under FR light condition, HY5 is a positive regulator for

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BBX14 expression (Figure 1B). In contrast, BBX14 is a negative regulator of *HY5* transcript expression in the dark without MeJA treatment (Figure 10A). However, under FR light, BBX14 is also a negative regulator of *HY5* transcript expression (Figure 10B). However, MeJA presence under FR light alters BBX14 function as a positive regulator in regulating *HY5* transcript expression (Figure 10B). Additionally, the *BBX14/PGRHY5* overexpression line is similar to Col-0 (Figure 8A), suggesting that *BBX14* overexpression results in a suppression of HY5 function under FR light.

BBX14 and COP1 show a genetic interaction in regulating hypocotyl elongation

Our quantitative real-time PCR analysis shows that the expression levels of *BBX14* in the *cop1-6* mutant are not significantly different from the wild type (Figure 1). Additionally, COP1 does not interact with BBX14 (Figure 5). However, the phenotype in *bbx14cop1-6* and *BBX14/cop1-6* reveal that COP1 acts as an extragenic suppressor of BBX14 in regulating hypocotyl elongation and COP1 is in the downstream of BBX14 in the control of seedling development in response to light and JA conditions (Figure 9). The regulatory relationship between BBX14 and COP1 in light and JA signaling remains elusive.

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Figures

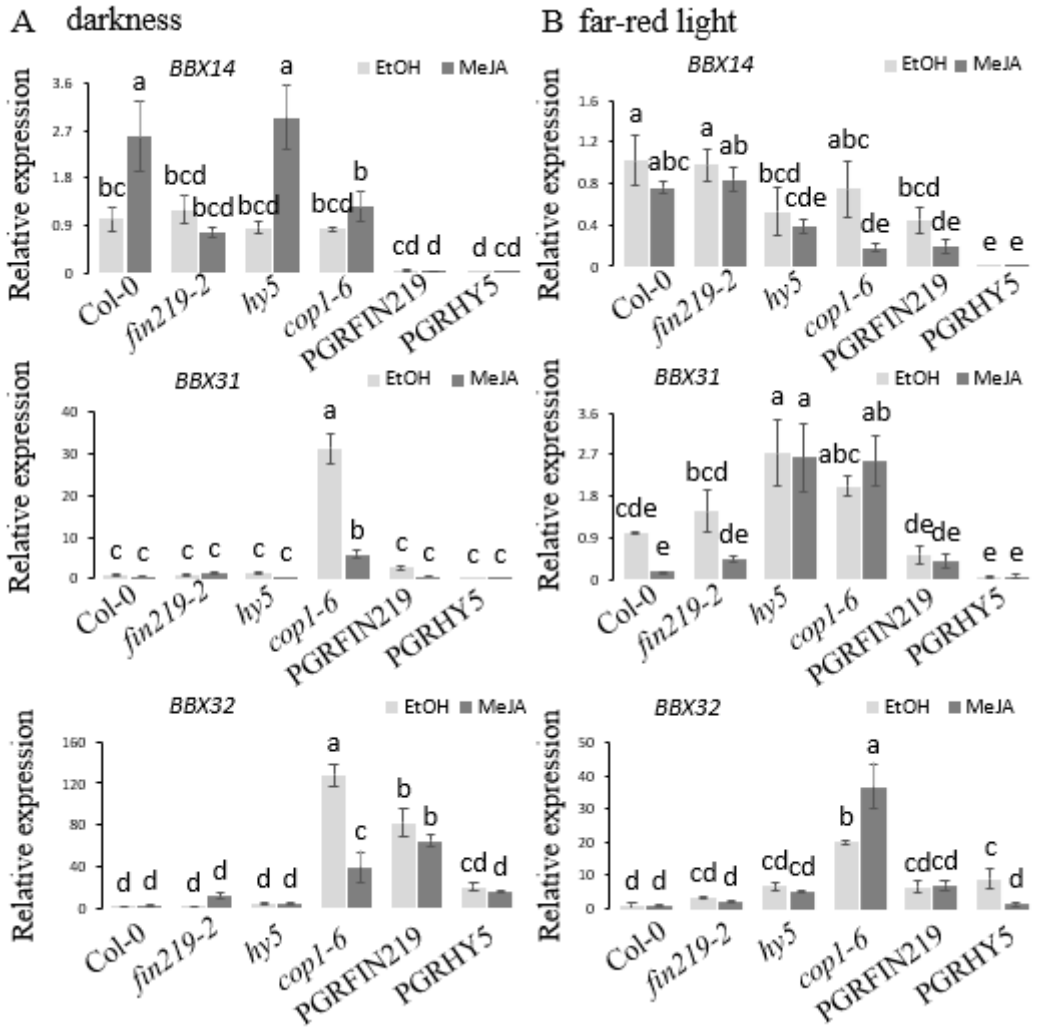

Figure 1. Regulation of *BBX14, BBX31*, and *BBX32* genes expression by FIN219, HY5, and COP1 in response to light and MeJA treatments. Quantitative real-time PCR analysis of BBX14, BBX31, and BBX32 relative expression in Col-0, *fin219-2, hy5* and *cop1-6* mutants, PGRFIN219, and PGRHY5 seedlings. (A) Under the darkness. (B) Under the far-red light 2 μmol m^{-2} s^{-1}. Seedlings were grown on MS medium without or with 50 μM MeJA, and 10 μM DEX was added to Col-0, PGRFIN219 and PGRHY5 seedlings. All seedlings were kept in the dark or far-red light and were 3 days old.

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UBQ10 was used as an internal control. Different lowercase letters indicate significant differences identified by one-way ANOVA with post-hoc Tukey test at $p<0.05$. Biological replicate done once.

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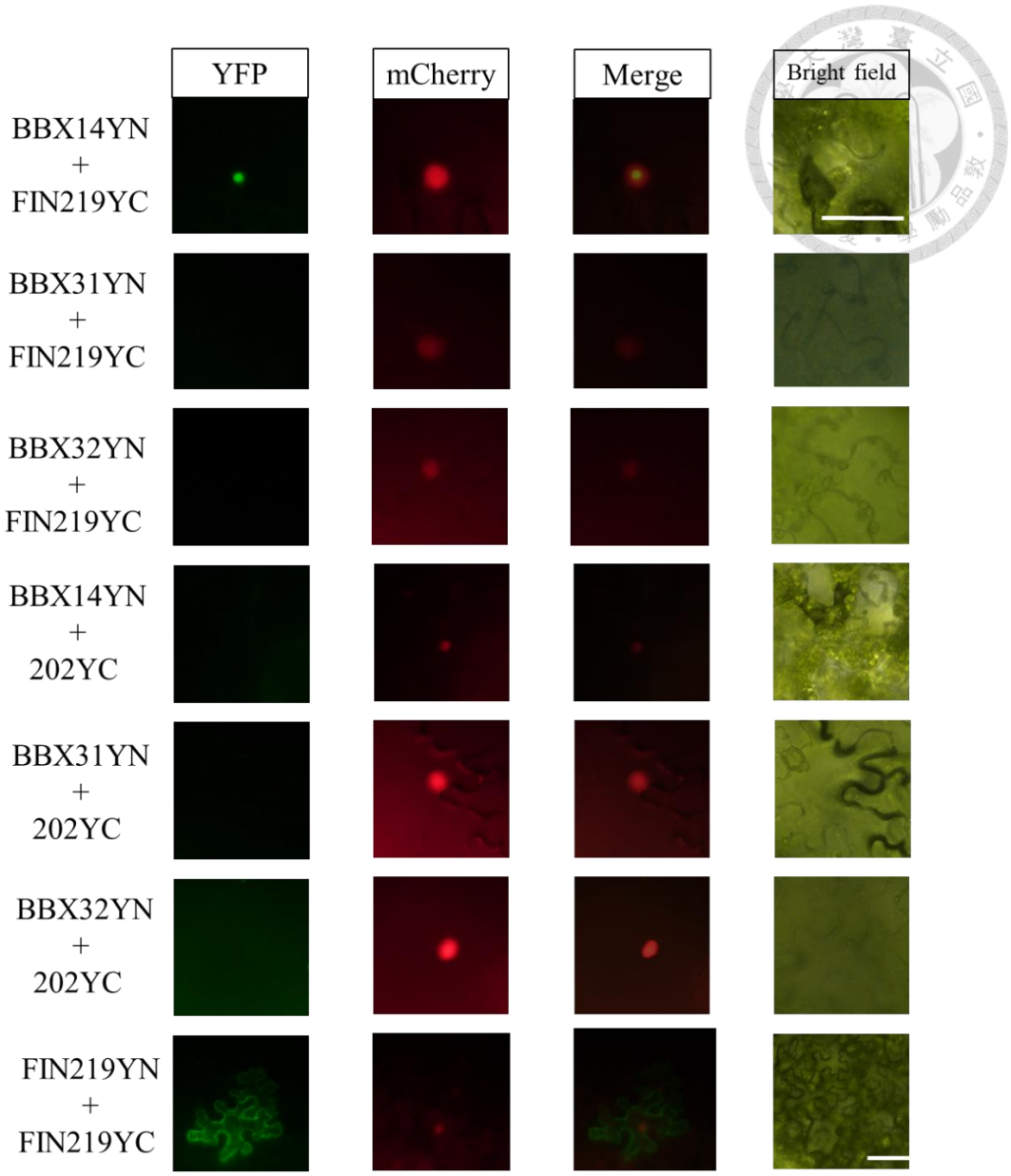

Figure 2. The candidate genes BBX14, BBX31, and BBX32 were tested for interactions with FIN219 under white light conditions. Empty vector was used as negative controls, while FIN219YN and FIN219YC were used as positive controls. After infiltration, the leaves were allowed to recover for 2 days under white light before being examined using confocal microscopy. NLS-mcherry was used as a nuclear marker. Bar = 50 μm.

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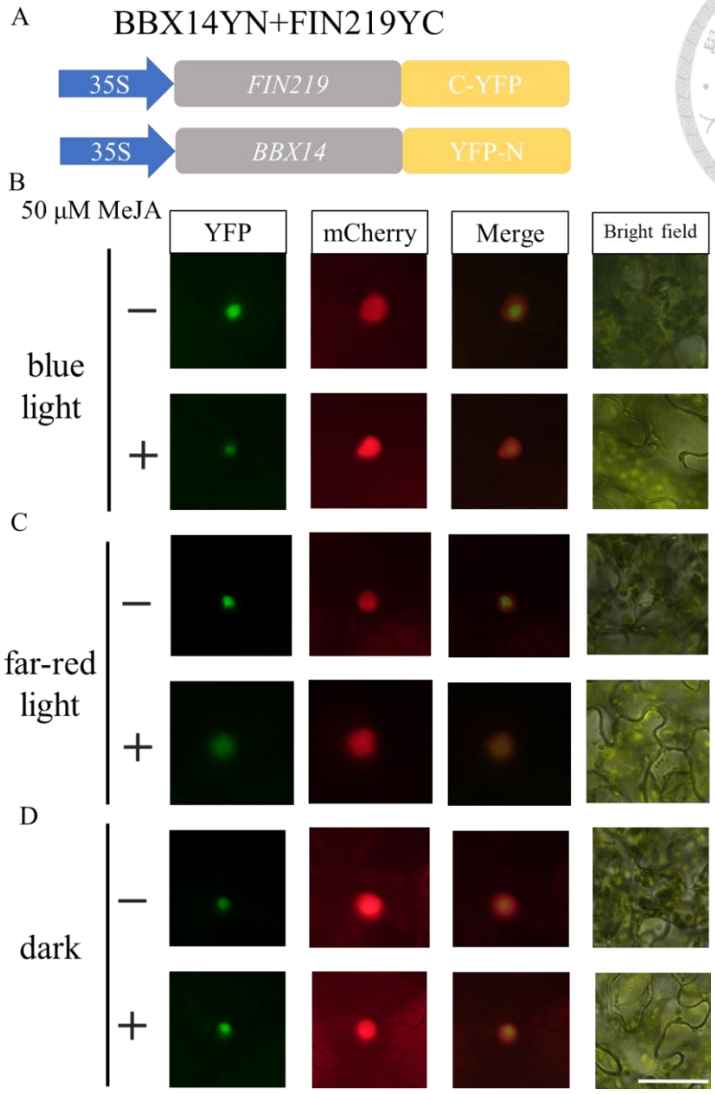

Figure 3. In the BiFC assay, it was observed that BBX14 interacts with FIN219 under far-red light, blue light, and dark conditions, regardless of the presence of MeJA. (A) Interaction observed using the BBX14YN construct and FIN219YC construct. (B) Continuous blue light at 1 μmol m^{-2} s^{-1}. (C) Continuous far-red light at 10 μmol m^{-2} s^{-1}. (D) Darkness. These interactions were identified using BiFC assays conducted in tobacco leaves, treated with or without 50 μM MeJA for 12 hours. Bar = 50 μm.

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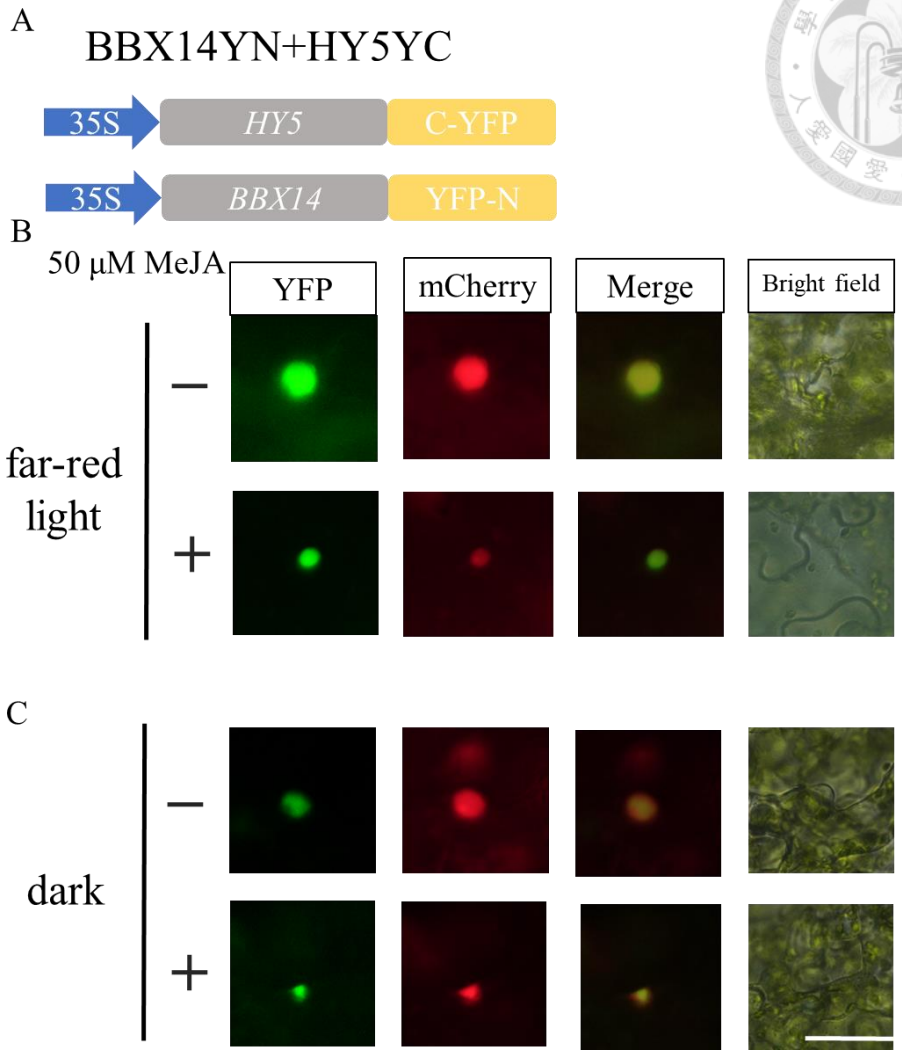

Figure 4. In the BiFC assay, it was observed that BBX14 interacts with HY5 under far-red and dark conditions, regardless of the presence of MeJA. (A) Interaction observed using the BBX14YN construct and HY5YC construct. (B) Continuous far-red light at 10 μmol m^{-2} s^{-1}. (C) Darkness. These interactions were identified using BiFC assays conducted in tobacco leaves, treated with or without 50 μM MeJA for 12 hours. Bar = 50 μm.

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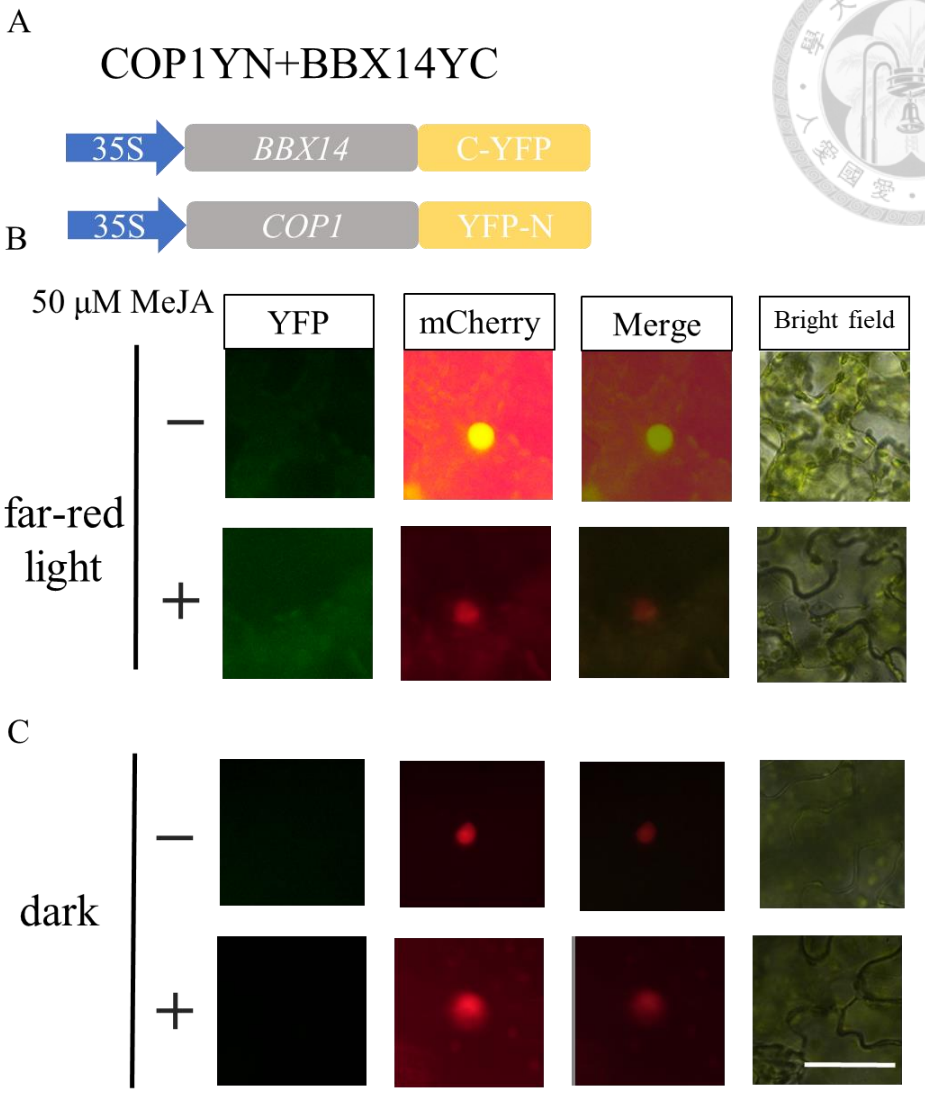

Figure 5. In the BiFC assay, it was observed that BBX14 did not interact with COP1 under far-red and dark conditions, regardless of the presence of MeJA. (A) Interaction observed using the COP1YN construct and BBX14YC construct. (B) Continuous far-red light at 10 μmol m^{-2} s^{-1}. (C) Darkness. These interactions were identified using BiFC assays conducted in tobacco leaves, treated with or without 50 μM MeJA for 12 hours. Bar = 50 μm.

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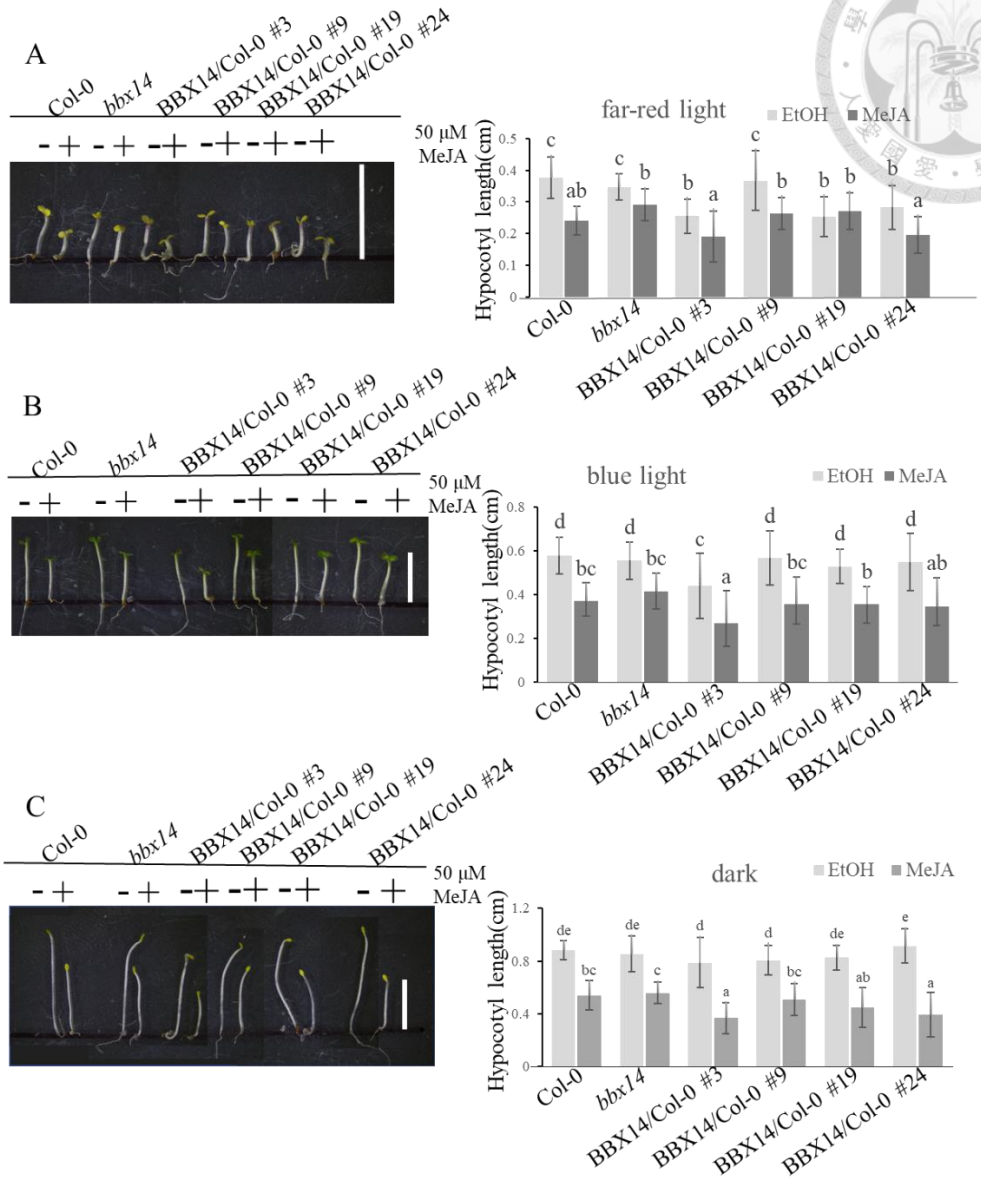

Figure 6. The *BBX14* overexpression line in the Col-0 background exhibits the hypocotyl length phenotype. The hypocotyl length phenotype was assessed in 3-day-old seedlings treated with 50 μM MeJA or without MeJA under (A) far-red light 2 μmol m^{-2} s^{-1}, (B) blue light 1 μmol m^{-2} s^{-1} or (C) dark conditions. Different letters indicate significant differences determined by one-way ANOVA with post-hoc Tukey test. Bar = 0.5 cm. Biological replicate done once.

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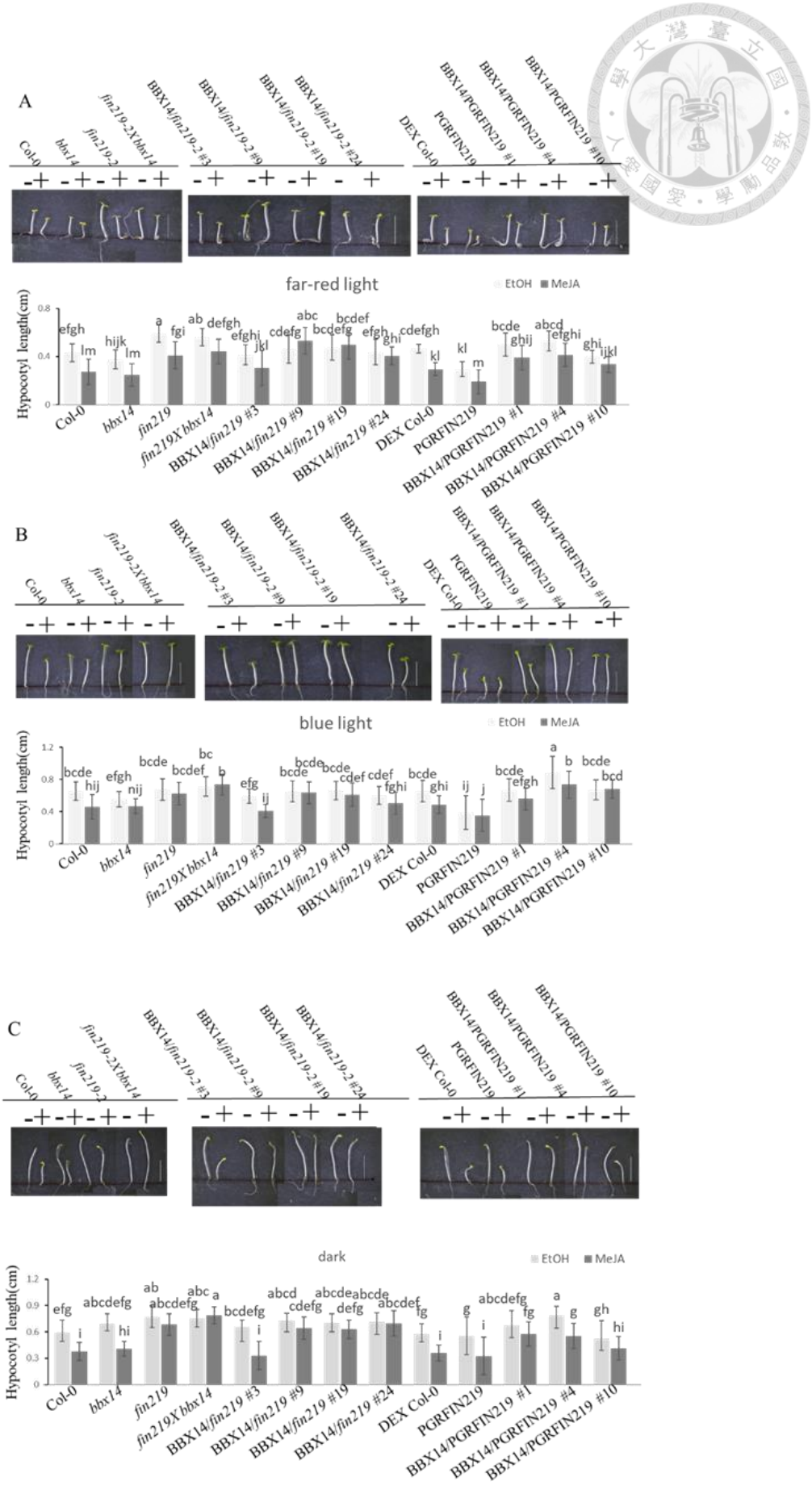

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Figure 7. The *BBX14* overexpression line in the *fin219-2* and *PGRFIN219* background exhibits the hypocotyl length phenotype. The hypocotyl length phenotype was assessed in 3-day-old seedlings treated with 50 μM MeJA or without MeJA under (A) far-red light 2 μmol m^{-2} s^{-1}, (B) blue light 1 μmol m^{-2} s^{-1} or (C) dark conditions. Different letters indicate significant differences determined by one-way ANOVA with post-hoc Tukey test. Bar = 0.5 cm. Biological replicate done once.

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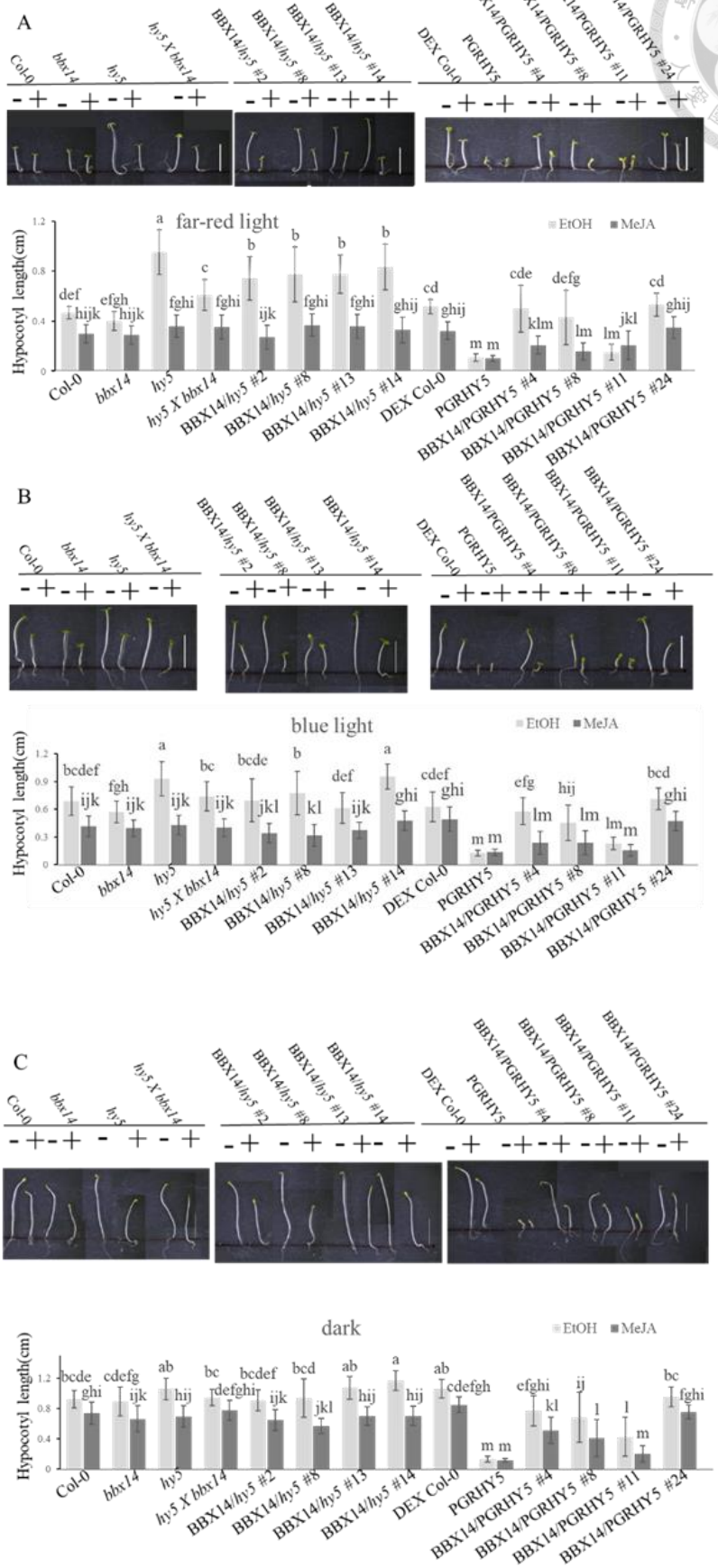

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Figure 8. The *BBX14* overexpression line in the *hy5* and *PGRHY5* background exhibits the hypocotyl length phenotype. The hypocotyl length phenotype was assessed in 3-day-old seedlings treated with 50 μM MeJA or without MeJA under (A) far-red light 2 μmol m^{-2} s^{-1}, (B) blue light 1 μmol m^{-2} s^{-1} or (C) dark conditions. Different letters indicate significant differences determined by one-way ANOVA with post-hoc Tukey test. Bar = 0.5 cm. Biological replicate done once.

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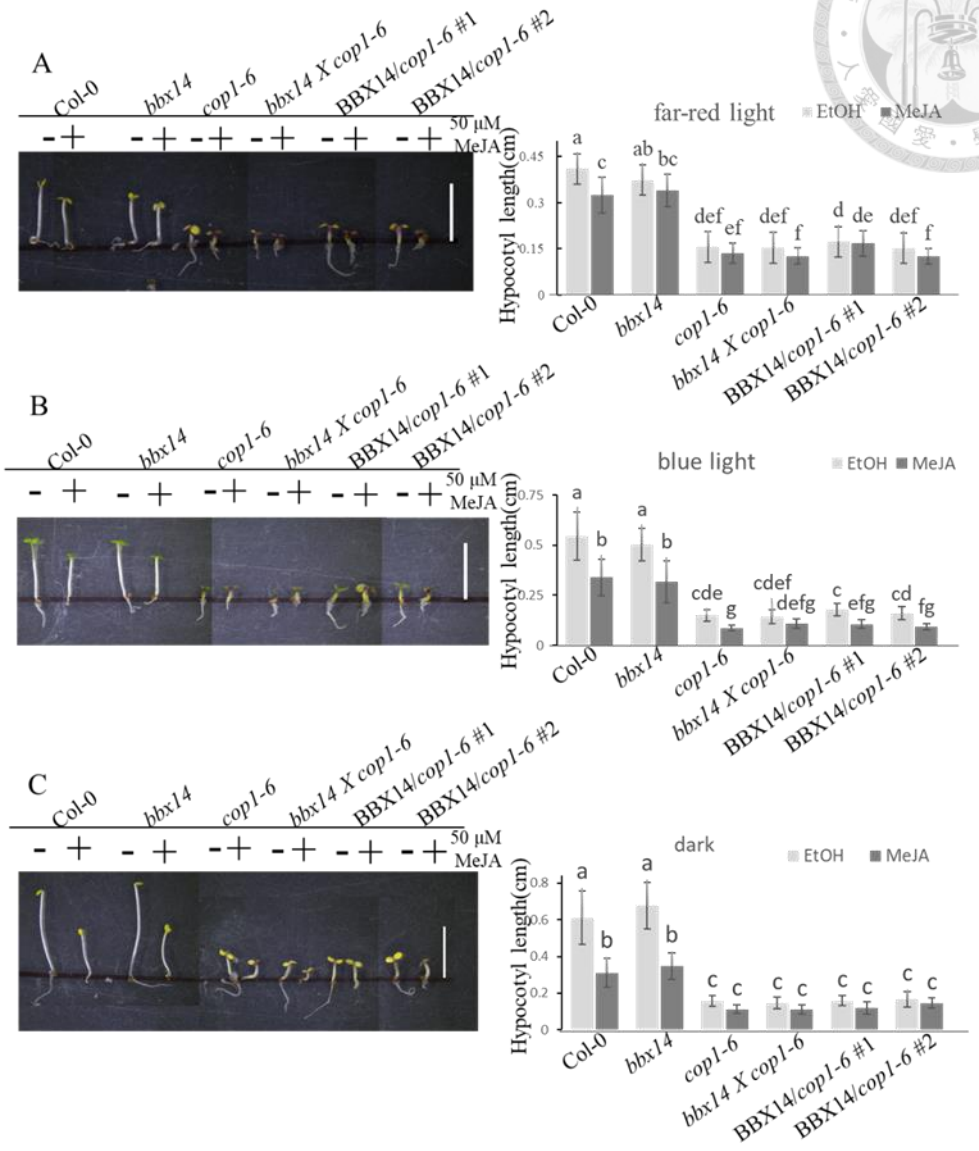

Figure 9. The *BBX14* overexpression line in the *cop1-6* background exhibits the hypocotyl length phenotype. The hypocotyl length phenotype was assessed in 3-day-old seedlings treated with 50 μM MeJA or without MeJA under (A) far-red light 2 μmol m^{-2} s^{-1}, (B) blue light 1 μmol m^{-2} s^{-1} or (C) dark conditions. Different letters indicate significant differences determined by one-way ANOVA with post-hoc Tukey test. Bar = 0.5 cm. Biological replicate done once.

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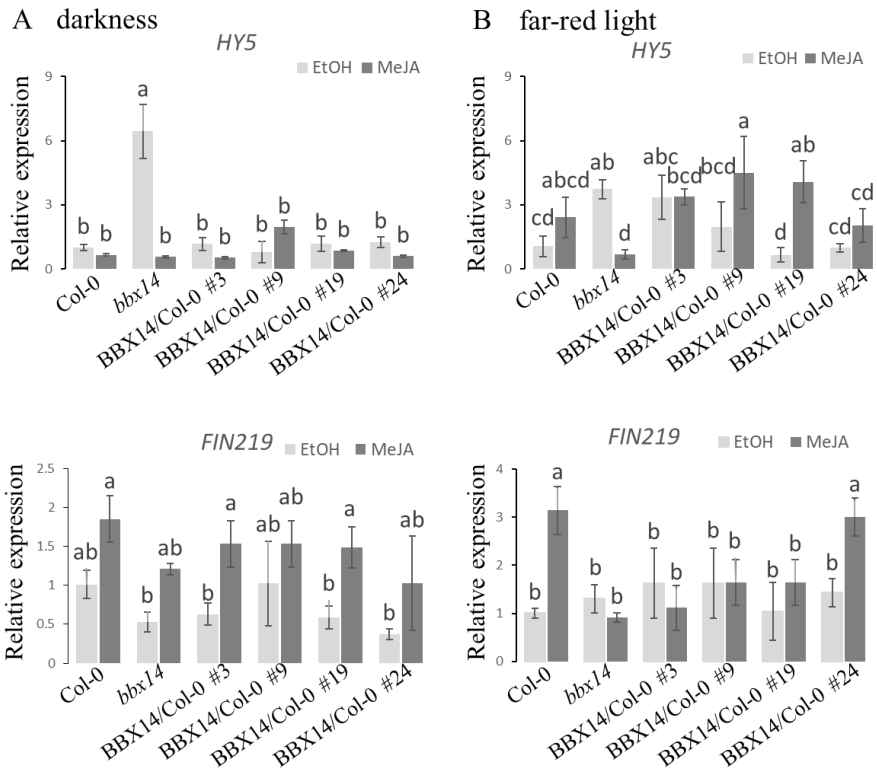

Figure 10. Quantitative real-time PCR analysis of *HY5* and *FIN219* relative expression in Col-0, *bbx14, BBX14*/Col-0 #3, #9, #19, and #24 seedlings. (A) under the darkness. (B) under the far-red light 2 μmol m^{-2} s^{-1}. Seedlings were grown on MS medium without or with 50 μM MeJA. All seedlings were kept in the dark or far-red light and were 3 days old. *UBQ10* was used as an internal control. Different lowercase letters indicate significant differences identified by one-way ANOVA with post-hoc Tukey test at $p<0.05$. Biological replicate done once.

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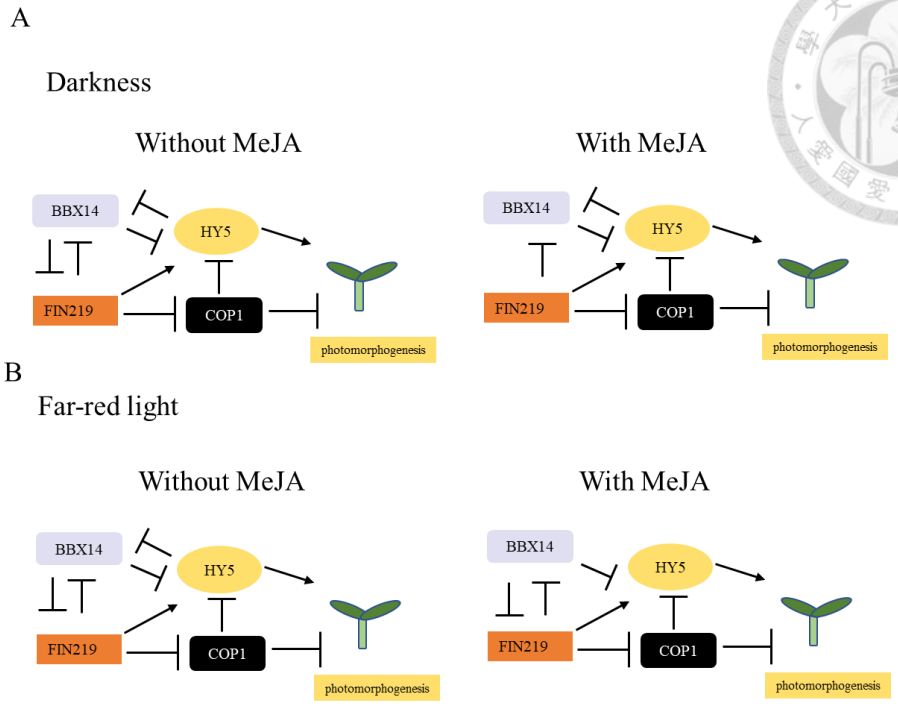

Figure 11. A model of interactions in the dark and far-red light with or without MeJA treatment. (A) Under dark conditions without MeJA treatment, BBX14 inhibits HY5, promoting hypocotyl elongation, possibly by directly binding to the *HY5* promoter to inhibit its transcription. BBX14 is also suppressed by HY5. Additionally, BBX14 possibly inhibits FIN219, promoting hypocotyl elongation, and is possibly suppressed by FIN219. With MeJA treatment, BBX14 continues to inhibit and be suppressed by HY5 and is also suppressed by FIN219. (B) Under far-red light conditions without MeJA treatment, BBX14 inhibits HY5, promoting hypocotyl elongation, possibly by directly binding to the *HY5* promoter to inhibit its transcription, while BBX14 possibly inhibits and is possibly suppressed by FIN219. With MeJA treatment, BBX14 continues to inhibit both HY5 and FIN219 and is also suppressed by FIN219.

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Supplemental Tables and Figures

Table S1. FIN219-regulated B-box (BBX) protein family in microarray data. The *fin219-2* mutant and *PGR219* (an inducible FIN219 overexpression line) affect the up- and down-regulation of several BBX transcription factors under low far-red (FR) light conditions with or without MeJA treatment, respectively. Wild-type seedlings, along with *fin219-2* mutant and *PGR219* lines, were cultivated under low FR light for 3 days, with or without 50 μM MeJA, and subsequently subjected to microarray analysis. A fold change threshold of ≥2 or ≤0.5.

PGR219/Col			***fin219-2*/Col**			**+MeJA*fin219-2*/Col**		
At3g21890	BBX31	0.06	At1g73870	BBX16	0.27	At3g21890	BBX31	0.34
At1g73870	BBX16	0.07	At3g21150	BBX32	0.37	At3g21150	BBX32	0.42
At4g27310	BBX28	0.28	At3g21890	BBX31	0.39	At5g54470	BBX29	2.53
At1g25440	BBX15	0.31				At5g57660	BBX6	2.15
At1g68520	BBX14	0.34				At1g49130	BBX17	2.03
At5g54470	BBX29	0.35						
At4g39070	BBX20	0.39						
At1g68190	BBX27	0.46						
At2g47890	BBX11	0.48						
At4g38960	BBX19	0.49						

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Table S2. qPCR primer list.

Name	Primer Number	Sequence	Tm °C
UBQ10-FQ	P1379	TCCGGATCAGCAGAGGCTTA	53.8
UBQ10-RQ	P1380	TCAGAACTCTCCACCTCAAG	51.8
FIN219-qPCR-F	P1386	TCCGGATCAGCAGAG GCTTA	53.7
FIN219-qPCR-R	P1387	TCAGAACTCTCACCTCAAG	51.7
qRT-Hy5 F	P2045	AAAGGCTTGCATCAGCATTAG	62.3
qRT-Hy5 R	P2046	GCGACTCTCTTACTCTTCAGAAC	63.3
BBX14 FOR qPCR F		AAGGCCTCGCATGAAAGGAAGG T	57.1
bbx14-3 UTR-R		CATACGTAGCTCACAAAAAC	47.7
BBX31-F		TCTTTCCTCAATATCACCCAGA	51.1
BBX31 FOR qPCR R		AAAGCTCACACCTTACCGGAA	52.4
BBX32 FOR qPCR F		ACGACGTGGCAGAATTTAAA	47.7
BBX32-R		TGCGGGACCCATGTCAATAAT	52.4

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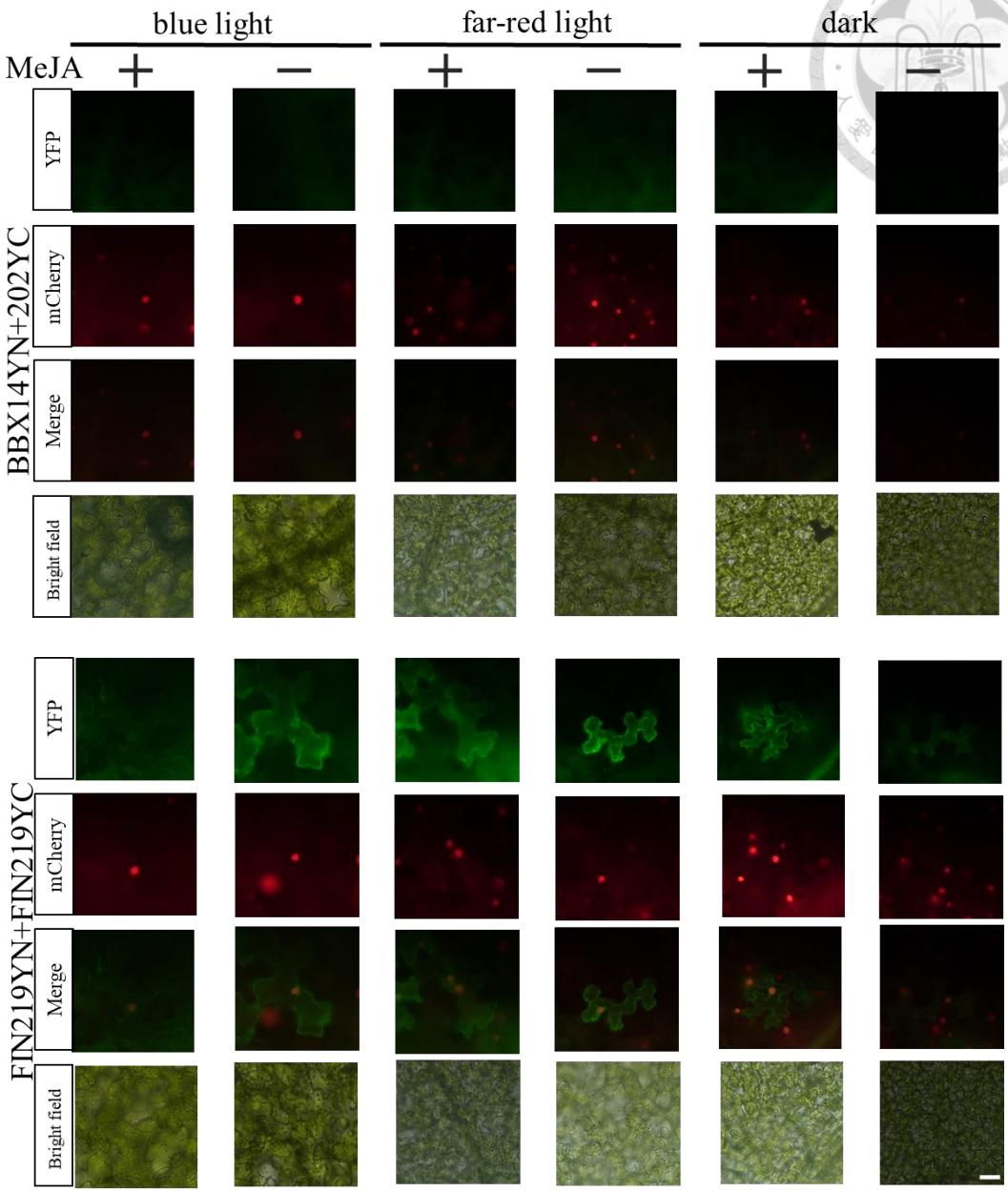

Figure S1. Different light conditions for the negative and positive control. Empty vector 202YC paired with BBX14YN was used as a negative control, while FIN219YN and FIN219YC were used as positive controls. Interactions were identified under blue light at 1 μmol m^{-2} s^{-1}, far-red light at 10 μmol m^{-2} s^{-1}, and darkness. These interactions were identified using BiFC assays conducted in tobacco leaves, treated with or without 50 μM MeJA for 12 hours. Bar = 50 μm.

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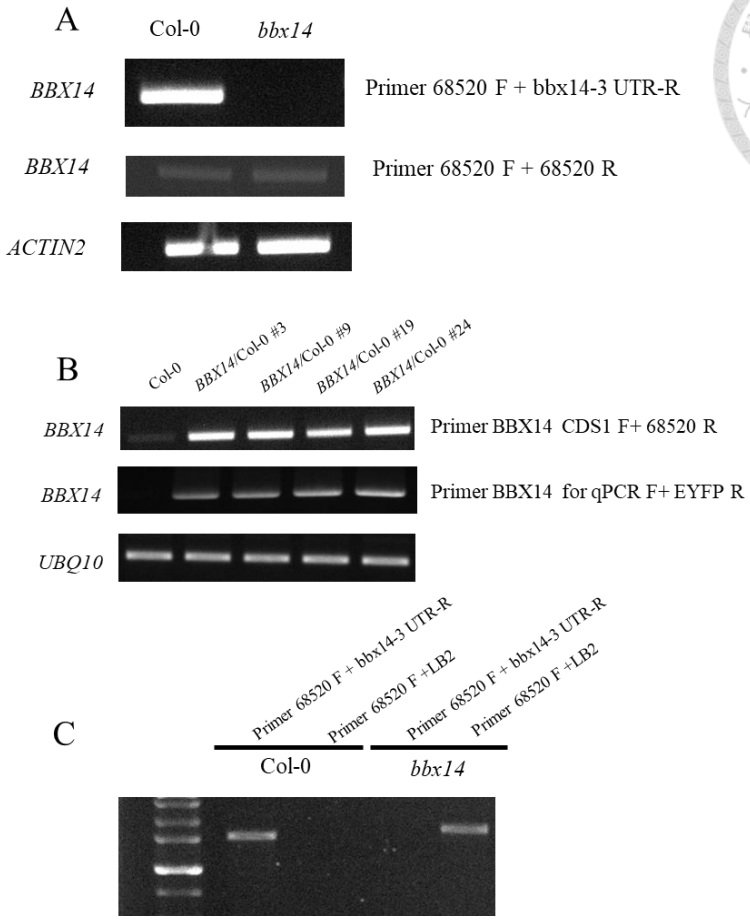

Figure S2. Examination of the insertion position of the *bbx14* mutant and analysis of the *BBX14* transcription levels in the *bbx14* mutant and *BBX14* overexpression lines. (A) Gene expression in the *bbx14* mutant. The top row shows the T-DNA insertion. The middle row shows the absence of the T-DNA insertion. Bottom row *ACTIN2* was used as the loading control. (B) Gene expression in the *BBX14* overexpression line. The top row shows the expression using the *BBX14* gene primer. The middle row shows the expression using both the *BBX14* and *EYFP* primers. Bottom row *UBQ10* was used as

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the loading control. (C) Genotyping of the *bbx14* mutant. Col-0 was used as the control in the first and second lanes. The *bbx14* mutant plant was used in the third and fourth lanes. The first and third lane used the *BBX14* primer, and the second and fourth lane used both the *BBX14* primer and the T-DNA insertion primer.

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